

gradients or chromatographic columns of cell lysates or proteins obtained from cell lysates and the use of GLUTX to identify proteins in the lysate that interact with GLUTX.

For these assays, the GLUTX polypeptide can be a full length 5 GLUTX, an extracellular domain of GLUTX, or some other suitable GLUTX polypeptide. Once isolated, such an interacting protein can be identified and cloned and then used, in conjunction with standard techniques, to alter the activity of the GLUTX polypeptide with which it interacts.

10 For example, at least a portion of the amino acid sequence of a protein that interacts with GLUTX can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique. The amino acid sequence obtained can be used as a guide for the generation 15 of oligonucleotide mixtures that can be used to screen for gene sequences encoding the interacting protein. Screening can be accomplished, for example, by standard hybridization or PCR techniques. Techniques for the generation of oligonucleotide mixtures and the screening are well-known 20 (Ausubel, *supra*; and "PCR Protocols: A Guide to Methods and Applications," Innis *et al.*, eds. Academic Press, Inc., NY, 1990).

Additionally, methods can be employed that result directly in the identification of genes that encode proteins 25 that interact with GLUTX. These methods include, for example, screening expression libraries, in a manner similar to the well known technique of antibody probing of λ gt11 libraries, using labeled GLUTX polypeptide or a GLUTX fusion protein, for example, a GLUTX polypeptide or domain fused to 30 a marker such as an enzyme, fluorescent dye, a luminescent protein, or to an IgFc domain.

There are also methods available that can detect protein-protein interaction *in vivo*. A method which detects

protein interactions *in vivo* is the two-hybrid system (Chien et al., *Proc. Natl. Acad. Sci. USA* 88:9578, 1991). A kit for practicing this method is available from Clontech (Palo Alto, CA).

5 Briefly, utilizing such a system, plasmids are constructed that encode two hybrid proteins: one plasmid includes a nucleotide sequence encoding the DNA-binding domain of a transcription activator protein fused to a nucleotide sequence encoding GLUTX, a GLUTX polypeptide, or
10 a GLUTX fusion protein, and the other plasmid includes a nucleotide sequence encoding the transcription activator protein's activation domain fused to a cDNA encoding an unknown protein which has been recombined into this plasmid as part of a cDNA library. The DNA-binding domain fusion
15 plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (e.g., HBS or LacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the
20 reporter gene: the DNA-binding domain hybrid cannot because it does not provide activation function, and the activation domain hybrid cannot because it cannot localize to the activator's binding sites. Interaction of the two hybrid proteins reconstitutes the functional activator protein and
25 results in expression of the reporter gene, which is detected by an assay for the reporter gene product.

The two-hybrid system or related methodology can be used to screen activation domain libraries for proteins that interact with the "bait" gene product. By way of example, 30 and not by way of limitation, GLUTX may be used as the bait gene product. Total genomic or cDNA sequences are fused to the DNA encoding an activation domain. This library and a plasmid encoding a hybrid of bait GLUTX gene product fused

to the DNA-binding domain are co-transformed into a yeast reporter strain, and the resulting transformants are screened for those that express the reporter gene. For example, a bait GLUTX gene sequence, such as that encoding 5 GLUTX or a domain of GLUTX can be cloned into a vector such that it is translationally fused to the DNA encoding the DNA-binding domain of the GAL4 protein. These colonies are purified and the library plasmids responsible for reporter gene expression are isolated. DNA sequencing is then used 10 to identify the proteins encoded by the library plasmids.

A cDNA library of the cell line from which proteins that interact with bait GLUTX gene product are to be detected can be made using methods routinely practiced in the art. According to the particular system described 15 herein, for example, the cDNA fragments can be inserted into a vector such that they are translationally fused to the transcriptional activation domain of GAL4. This library can be co-transformed along with the bait GLUTX gene-GAL4 fusion plasmid into a yeast strain which contains a lacZ gene 20 driven by a promoter which contains GAL4 activation sequence. A cDNA encoded protein, fused to GAL4 transcriptional activation domain, that interacts with bait GLUTX gene product will reconstitute an active GAL4 protein and thereby drive expression of the HIS3 gene. Colonies 25 that express HIS3 can then be purified from these strains and used to produce and isolate the bait GLUTX gene-interacting protein using techniques routinely practiced in the art.

30 **IX. Detection of GLUTX or Nucleic Acid Molecules
Encoding GLUTX and Related Diagnostic Assays**

The invention encompasses methods for detecting the presence of GLUTX protein or nucleic acid in a biological